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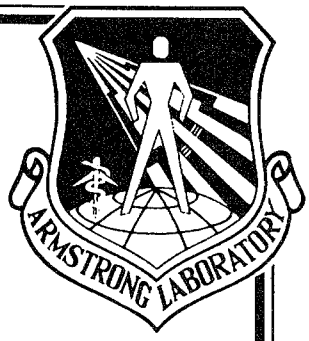
VOLUME I of IV



ARMSTRONG LABORATORY

GENETIC TOXICITY EVALUATION OF 1, 3, 3-TRINITROAZETIDINE

VOLUME I: RESULTS OF *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY

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February 1994

FINAL REPORT FOR THE PERIOD JULY THROUGH DECEMBER 1992

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TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0069

VOLUME I

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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13. ABSTRACT (Maximum 200 words)

The *Salmonella typhimurium* reverse mutation assay (Ames assay) evaluated the potential of 1,3,3-trinitroazetidine (TNAZ) to induce histidine (his) reversion (his^- to his^+), caused by base changes or frameshift mutations in the genome of this organism. This direct plate incorporation assay was conducted with five strains of *Salmonella typhimurium*, in the presence and absence of an exogenous mammalian activation system. The preincubation technique was used to enhance the sensitivity o the plate incorporation assay. The range finding study was conducted with and without metabolic activation to determine levels at which TNAZ exhibited toxicity. The reverse mutation assay determined the test substance to be non-mutagenic. The results of the assay were confirmed through an independent confirmatory assay.

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PREFACE

1,3,3-Trinitroazetidine (TNAZ) (CAS No. 97645-24-4) is a highly energetic castable explosive that is being considered by the Department of Defense for military and space applications. As a candidate replacement for select explosives, toxicity information is needed. A comprehensive literature search indicated that no information was available on the mutagenic potential of TNAZ. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of TNAZ. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing Guidelines, 40 CFR, Part 798 (7-1-90 edition).

This document, Volume I of IV, serves as a final report detailing the results of the *salmonella typhimurium* reverse mutation assay (Ames assay) in the genetic toxicity evaluation of TNAZ. Volumes II and III will describe, respectively, the results of the mouse bone marrow micronucleus test and the results of gene mutation at the HGPRT locus in cultured Chinese hamster ovary cells. Volume IV will serve as a summary report presenting the pertinent findings of the three assays described in Volumes I through III.

The research described herein began in July 1992 and was completed in December 1992 by the Toxikon Corporation, Woburn, MA, under a subcontract to ManTech Environmental Technology Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F19). Lt Col James N. McDougal served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

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TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

1.0 SUMMARY

The *Salmonella typhimurium* Reverse Mutation Assay (Ames Assay) evaluated the potential of the test substance to induce histidine (his) reversion (his^- to his^+), caused by base changes or frameshift mutations in the genome of this organism. This direct plate incorporation assay was conducted with five strains of *Salmonella typhimurium*, in the presence and absence of an exogenous mammalian activation system. The preincubation technique was used to enhance the sensitivity of the plate incorporation assay. The Range Finding Study was conducted with and without metabolic activation to determine levels at which the test substance exhibited toxicity. The Reverse Mutation Assay determined the test substance to be non-mutagenic. The results of the assay were confirmed through an independent Confirmatory Assay. The test substance, 1,3,3-Trinitroazetidine (TNAZ), meets with the criteria of the study protocol.

2.0 PURPOSE

The *Salmonella typhimurium* Reverse Mutation Assay (Ames Assay) evaluated the potential of a test substance to induce histidine (his) reversion (his^- to his^+), caused by base changes or frameshift mutations in the genome of this organism. This direct plate incorporation assay was conducted with five strains of *Salmonella typhimurium*, in the presence and absence of an exogenous mammalian activation system, after a preincubation period defined in the protocol.

3.0 MANAGEMENT OF THE STUDY

3.1 Sponsor: ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437

Project Officer: Darol Dodd, Ph.D.

3.2 Testing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Study Director: Inder J. Paika, Ph.D.

Quality Assurance: Kathryn M. Balch, B.A.

4.0 TECHNICAL REFERENCES

The study was conducted based on TSCA 40 CFR, Part 798, Subpart F, Section 798.5265; and Ames *et al*, Mutation Res., 31: 347-364, 1975.

5.0 COMPLIANCE

The study conformed to all applicable laws and regulations. Specific regulatory requirements included the current Good Laboratory Practice Standards, TSCA (EPA) 40 CFR, Part 792.

6.0 TEST SUBSTANCE

The following information was supplied by the Sponsor wherever applicable. Confidential information did not apply. The Sponsor was responsible for all test substance characterization data as specified in the GLP regulations.

Test Substance Name: 1,3,3-Trinitroazetidine (TNAZ)
Lot/Batch #: Not Supplied by Sponsor (N/S)
CAS/Code #: 97645-24-4
Physical State: White Granular Solid
Color: White
Density: 1.84
pH: N/S
Stability: Class A Explosive
Solubility: Negligible in Water; DMSO
Source: Eglin AFB, FL 32542-5000
Storage Conditions: 0^{o} to 120^{o}F
Safety Precautions: Special Safety Precautions for Class A Explosive

7.0 JUSTIFICATION OF THE TEST SYSTEM

Historically, the Reverse Mutation Assay in *Salmonella typhimurium* has been used to detect mutation in a gene of a histidine requiring strain to produce a histidine independent strain of this organism. This test system is recommended in TSCA 40 CFR, Part 798, Subpart F, Section 798.5265; and Ames *et al*, Mutation Res., 31: 347-364, 1975.

8.0 IDENTIFICATION OF THE TEST SYSTEM

The *Salmonella typhimurium* strains used in this assay were TA98, TA100, TA1535, TA1537 and TA1538. These strains were received directly from Carol Wehr, Department of Biochemistry, Room 420, University of California, Berkley, CA 94720.

9.0 JUSTIFICATION OF TEST SUBSTANCE ADMINISTRATION ROUTE

The test substance was administered *in vitro*, directly to the test system. This was the only route of administration available in this test system.

10.0 EXPERIMENTAL DESIGN

10.1 Preincubation:
S9 Mix or Phosphate Buffer (0.5 mL) were aliquoted into sterile

capped culture tubes placed in an ice bath. The tester strain (0.1 mL) and the test substance (dissolved in DMSO) was added to each tube. The tubes were vortexed gently and incubated at 37±2°C for 20±2 minutes prior to plating. The control substances were treated similar to the test substance.

10.2 Method:
Added to each tube of the preincubated suspension of test substance and tester strain, treated with and without a metabolic activation system, was Top Agar supplemented with histidine-biotin solution. The tubes were directly plated on a minimal medium. After a period of incubation, revertant colonies will be counted and compared to the number of spontaneous revertants in a negative control substance culture.

10.3 Tester Strains:
The requirement of histidine for growth were demonstrated for each *Salmonella* strain. Other phenotypic characteristics were verified by crystal violet sensitivity and resistance to ampicillin. Spontaneous reversion frequency was within the range expected either as reported in literature, or as established by Toxikon's historical mean values.

10.4 Stock Cultures:
Working stock cultures were grown fresh for the assay. Frozen stock for each strain was thawed and inoculated into sterile nutrient broth. Cultures were incubated overnight at 37±2°C. Cultures with an Absorbance >0.5 at 650 nm, read against a nutrient broth blank, were used in the assay. They were refrigerated or kept on ice until the start of the assay, and then maintained at room temperature during the assay.

10.5 Solubility:
The test substance, a powder, was dissolved in dimethylsulfoxide (DMSO). The Sponsor indicated that the test article was 100% soluble in acetone, as well as DMSO. No other test substance preparation was specified by Sponsor.

10.6 Negative Control Substance:

Tester strains were preincubated with the appropriate solvent at the corresponding maximum concentration, and plated with and without metabolic activation. This served as the negative control substance, and provided reference for background lawns and revertant colony formation.

10.7 Positive Control Substances:
Tester strains were preincubated with the appropriate positive control substance and plated with and without metabolic activation.

10.7.1 Positive control substances without metabolic activation were strain specific:

TA98	2-Nitrofluorene	10	ug/mL
TA100	Sodium Azide	100	ug/mL
TA1535	Sodium Azide	5	ug/mL
TA1537	9-Aminoacridine	800	ug/mL
TA1538	2-Nitrofluorene	10	ug/mL

10.7.2 The positive control substance with metabolic activation was 2-Aminoanthracene for all strains.

TA98	2-Aminoanthracene	5	ug/mL
TA100	2-Aminoanthracene	10	ug/mL
TA1535	2-Aminoanthracene	20	ug/mL
TA1537	2-Aminoanthracene	30	ug/mL
TA1538	2-Aminoanthracene	10	ug/mL

10.7.3 The appropriate concentrations for all positive control substances was dosed at 100 ul/plate.

10.8 Replication:
Tester strains were treated with six levels of concentrations. All controls and test groups were plated in triplicate.

10.9 Non-activated Assay:
Top agar, supplemented with 0.5 mM histidine - 0.5 mM biotin per 1.0 ml of agar, was used as an overlay. The agar was maintained at 42-48^{o}C until use. The overlay consisted of sterile tubes containing:

2 ml of molten top agar
0.1 ml of the appropriate tester strain
0.1 ml of the appropriate concentration of the test substance or control substance
0.5 ml of Phosphate Buffer (pH=7.4) or S9 mix

Vortexed tubes were poured onto Minimal Glucose Agar plates. Plates were incubated at 37$\pm$2^{o}C for 48-72 hours, checked for uniform background lawns, and revertant colonies counted.

10.10 Metabolic Activation Assay:
Tubes requiring metabolic activation contained an S9 fraction of rat liver homogenate obtained from Aroclor^{R} 1254 treated Sprague Dawley rats. The S9 activation system, prepared fresh on the day of the assay and kept refrigerated or on ice, contained the following per 10 ml:

0.4 M $MgCl_2$/1.65M KCL	0.20 ml
1 M Glucose-6-Phosphate	0.05 ml
0.1 M NADP	0.40 ml
0.2 M Phosphate Buffer pH=7.4	5.00 ml
USP Water for Injection	3.35 ml
S9 Fraction	1.00 ml

The method of preparation for the top agar was the same as in the non-activated assay, with the exception of substituting the 0.5 ml complete S9 mix for the phosphate buffer.

11.0 DOSAGE

11.1 Range Finding Assay:
A Range Finding Assay was performed with and without metabolic activation to determine levels at which the test substance exhibited toxicity. The assay was conducted only with TA100 and negative control substance plates. The test was conducted over a broad range of concentrations. In determining the upper limits of test substance concentration, cytotoxicity and solubility were considered. The test substance was assayed at ten concentrations. Concentrations tested included 10000, 5000, 1000, 500, 100, 50, 10, 5, 1, 0.5 and 0.1 ug/plate. Since toxicity was detected, the concentrations tested in the Reverse Mutation Assay were chosen to bracket between toxic and non-toxic levels. Alternate concentrations were not requested by the Sponsor.

11.2 Reverse Mutation Assay:
The dose levels to be tested in the Reverse Mutation Assay were selected based on the results of the Range Finding Assay. Ideally, the highest dose should cause some toxicity. The Reverse Mutation Assay was conducted over a broad range of six concentrations. In determining the upper limits of test substance concentration, cytotoxicity and solubility were considered. Concentrations tested included 500, 50, 5, 0.5, 0.05 and 0.01 ug/plate. The test substance was soluble at all concentrations assayed. Alternative doses were not requested by the Sponsor.

12.0 EVALUATION CRITERIA

12.1 Evaluation Criteria of the Range Finding Assay:
The Range Finding Assay was performed with strain TA100, negative control substance plates, with and without microsomal activation. Toxicity was determined by a reduction in the number of spontaneous revertants, a clearing of the background lawn, or by the degree of survival of treated cultures.

The negative control substance plates give a reference point from which to compare the data. The negative control substance values should fall within two standard deviations of the historical mean value for Toxikon or reference literature.

The mean number of revertants per plate should be calculated for each concentration. A positive result for any strain is a significant increase over the negative control number of revertants per plate, which is concentration dependent. A significant increase is defined as at least a two-fold increase in the number of spontaneous revertant colonies on the test substance plates when compared to the number of revertant colonies on its corresponding negative control substance plates.

If toxicity was detected, dose levels should be chosen to bracket toxic and non-toxic levels.

12.2 Evaluation Criteria of the Reverse Mutation Assay:
The positive control substance assays consisted of direct-acting mutagens and mutagens requiring metabolic biotransformation. All positive controls must exhibit twice the number of colonies as the negative control substances, to demonstrate that the test system is functional with known mutagens. The negative control substance plates, for each strain, gives a reference point to compare the test data. If their values do not fall within two standard deviations of the historical or literature mean values, the remaining plates are not scored and the assay will be repeated.

For the test substance to be considered mutagenic, the number of revertant colonies associated with the test substance must represent at least a two-fold increase over the number of revertant colonies associated with the corresponding negative control substance. Results for a strain will be rejected if the positive control substance does not yield a mutagenic response or if the negative control substance values fall outside the 95% confidence limit of the historical background.

12.3 Dose Response Phenomena:
The demonstration of a dose-related increases in revertant counts is an important criterion in establishing mutagenicity. Since several dose levels were utilized in the actual assay, a dose response would normally be seen with a mutagenic test substance.

13.0 RESULTS

13.1 Range Finding Assay (Tables I and II):
The Range Finding Assay was performed with strain TA100, negative control substance plates, with and without microsomal activation. Some toxicity was observed, as determined by a reduction in the number of spontaneous revertants, a clearing of the background lawn, and by the degree of survival of treated cultures.

The negative control substance plates gave a reference point from which to compare the data. The negative control substance values fell within two standard deviations of the historical mean value for Toxikon or reference literature.

The mean number of revertants per plate was calculated for each concentration. A positive result was not observed for any strain since a significant increase in the number of revertant colonies over its corresponding negative control substance was not observed.

Since toxicity was detected, dose levels were chosen to bracket toxic and non-toxic levels.

13.2 Reverse Mutation Assay (Tables III and IV):
The positive control substance assays consisted of direct-acting mutagens and mutagens requiring metabolic biotransformation. All positive controls exhibited twice the number of colonies as the negative control substances, demonstrating that the test system was functional with known mutagens. The negative control substance plates, for each strain, gave a reference point to compare the test data. Their values fell within two standard deviations of the historical or literature mean values.

The test substance is not considered mutagenic because the number of revertant colonies associated with the test substance did not represent a two-fold increase over the number of revertant colonies associated with the corresponding negative control substance (Refer Tables III and IV). The results are considered valid since the positive control substance yielded a mutagenic response and the values for the negative control substance fell within the 95% confidence limit of the historical background.

13.3 Dose Response Phenomena:
A dose response was not observed for the test article in the Reverse Mutation Assay.

13.4 Confirmatory Assay:
The results of the assay were confirmed through an independent Confirmatory Assay (run on fresh sample), as requested by Sponsor (Tables V through VIII).

14.0 CONCLUSION

The *Salmonella typhimurium* Reverse Mutation Assay (Ames Assay) evaluated the potential of the test substance to induce histidine (his) reversion (his^- to his^+), caused by base changes or frameshift mutations in the genome of this organism. This direct plate incorporation assay was conducted with five strains of *Salmonella typhimurium*, in the presence and absence of an exogenous mammalian activation system. The preincubation technique was used to enhance the sensitivity of the plate incorporation assay. The Range Finding Study was conducted with and without metabolic activation to determine levels at which the test substance exhibited toxicity. The Reverse Mutation Assay determined the test substance to be non-mutagenic. The results of the assay were confirmed through an independent Confirmatory

Assay. The test substance, 1,3,3-Trinitroazetidine (TNAZ), meets with the criteria of the study protocol.

15.0 CONFIDENTIALITY

Statements of confidentiality were as agreed upon prior to study contract initiation.

16.0 RECORDS

Original Data:	Toxikon Corporation Archives
Final Report:	Toxikon Corporation Archives
Test Article:	Remaining test article will be returned to the Sponsor.

17.0 VERIFICATION DATA

Protocol Signature (Toxikon):	07/27/92
Project Log Date:	08/17/92
Range Finding Assay Technical Initiation:	09/22/92
Range Finding Assay Technical Completion:	09/25/92
Reverse Mutation Technical Initiation:	10/09/92
Reverse Mutation Technical Completion:	10/12/92
Confirmation Assay:-	
Range Finding Assay Technical Initiation:	11/06/92
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Reverse Mutation Technical Initiation:	11/12/92
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Final Report:	11/17/92
Revised Report:	12/11/92

18.0 SIGNATURE OF AUTHORIZED PERSONNEL

Inder J. Paika, Ph.D.
Study Director

12/11/92
Date

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

TABLE I

Range Finding Assay
Without Microsomal Activation

Technical Initiation: 09/22/92

Technical Completion: 09/25/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE				
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 100000	5000	1000	500	100
	NA	147	0	0	0	0	97
TA100	NA	139	0	0	0	0	86
	NA	136	0	0	0	0	89
MEAN	0.0	140.7	0.0	0.0	0.0	0.0	90.7
SD	0.0	5.7	0.0	0.0	0.0	0.0	5.7

STRAIN	Positive Control**	50	10	5	1	0.5	0.1
	NA	126	138	151	158	162	136
TA100	NA	145	150	153	160	152	151
	NA	149	146	145	149	155	150
MEAN	0.0	140.0	144.7	149.7	155.7	156.3	145.7
SD	0.0	12.3	6.1	4.2	5.9	5.1	8.4

* All plates were dosed at 100 ul/plate
** Positive controls were not used in the Range Finding Assay
*** The negative control used in the assay was DMSO

TABLE II

Range Finding Assay
With Microsomal Activation

Technical Initiation: 09/22/92

Technical Completion: 09/25/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE				
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 100000	5000	1000	500	100
TA100	NA	145	0	0	0	0	38
	NA	159	0	0	0	0	46
	NA	156	0	0	0	0	63
MEAN	0.0	153.3	0.0	0.0	0.0	0.0	49.0
SD	0.0	7.4	0.0	0.0	0.0	0.0	12.8

STRAIN	Positive Control**	50	10	5	1	0.5	0.1
TA100	NA	150	139	161	133	161	140
	NA	166	128	142	148	155	152
	NA	159	152	150	150	156	161
MEAN	0.0	158.3	139.7	151.0	143.7	157.3	151.0
SD	0.0	8.0	12.0	9.5	9.3	3.2	10.5

* All plates were dosed at 100 ul/plate
** Positive controls were not used in the Range Finding Assay
*** The negative control used in the assay was DMSO

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

TABLE III
Reverse Mutation Assay
Without Microsomal Activation

Technical Initiation: 10/09/92
Technical Completion: 10/12/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE					
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 500	50	5	0.5	0.05	0.01
	160	34	0	30	33	32	36	32
TA98	163	33	0	32	30	31	30	32
	175	34	0	31	34	34	31	30
MEAN	166.0	33.7	0.0	31.0	32.3	32.3	32.3	31.3
SD	7.9	0.6	0.0	1.0	2.1	1.5	3.2	1.2
	319	135	0	126	120	130	126	140
TA100	326	129	4	135	131	129	128	132
	343	143	1	128	130	125	129	129
MEAN	329.3	135.7	1.7	129.7	127.0	128.0	127.7	133.7
SD	12.3	7.0	2.1	4.7	6.1	2.6	1.5	5.7
	146	22	0	24	25	21	24	26
TA1535	155	24	0	22	20	23	22	21
	155	23	0	23	24	24	23	22
MEAN	152.0	23.0	0.0	23.0	23.0	22.7	23.0	23.0
SD	5.2	1.0	0.0	1.0	2.6	1.5	1.0	2.6
	104	12	0	10	13	10	12	13
TA1537	107	12	0	11	12	10	11	12
	105	13	0	11	13	9	14	10
MEAN	105.3	12.3	0.0	10.7	12.7	9.7	12.3	11.7
SD	1.5	0.6	0.0	0.6	0.6	0.6	1.5	1.5
	123	18	0	15	16	20	18	17
TA1538	127	19	0	19	17	18	18	15
	124	17	0	18	19	17	19	19
MEAN	124.7	18.0	0.0	17.3	17.3	18.3	18.3	17.0
SD	2.1	1.0	0.0	2.1	1.5	1.5	0.6	2.0

* All plates were dosed at 100 ul/plate

** The positive control used was sodium azide for strains TA-100 and TA-1535, 2-nitrofluorene for strain TA-98, and 9-aminoacridine for strain TA-1537

*** The negative control used in the assay was DMSO

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

TABLE IV
Reverse Mutation Assay
With Microsomal Activation

Technical Initiation: 10/09/92
Technical Completion: 10/12/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE					
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 500	50	5	0.5	0.05	0.01
	171	41	0	42	43	41	40	42
TA98	176	40	0	39	41	43	45	40
	176	40	0	40	40	44	42	39
MEAN	174.3	40.3	0.0	40.3	41.3	42.7	42.3	40.3
SD	2.9	0.6	0.0	1.5	1.5	1.5	2.5	1.5
	426	177	14	185	174	175	169	171
TA100	404	188	12	176	176	180	173	168
	434	180	9	179	172	177	170	176
MEAN	421.3	181.7	11.7	180.0	174.0	177.3	170.7	171.7
SD	15.5	5.7	2.5	4.6	2.0	2.5	2.1	4.0
	183	28	0	27	25	27	28	27
TA1535	190	23	0	27	24	26	25	26
	189	26	0	26	28	27	26	26
MEAN	187.3	25.7	0.0	26.7	25.7	26.7	26.3	26.3
SD	3.8	2.5	0.0	0.6	2.1	0.6	1.5	0.6
	126	12	0	15	13	14	13	15
TA1537	119	16	0	14	15	12	14	13
	125	15	0	12	15	12	14	14
MEAN	123.3	14.3	0.0	13.7	14.3	12.7	13.7	14.0
SD	3.8	2.1	0.0	1.5	1.2	1.2	0.6	1.0
	128	18	0	20	21	15	22	21
TA1538	136	21	0	20	18	16	20	23
	126	19	0	19	18	21	20	19
MEAN	130.0	19.3	0.0	19.7	19.0	17.3	20.7	21.0
SD	5.3	1.5	0.0	0.6	1.7	3.2	1.2	2.0

* All plates were dosed at 100 ul/plate
** The positive control used was 2-aminoanthracene for all strains
*** The negative control used in the assay was DMSO

TABLE V

Confirmation Assay - Range Finding
Without Microsomal Activation

Technical Initiation: 11/06/92

Technical Completion: 11/09/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE				
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 100000	5000	1000	500	100
	NA	169	0	0	0	0	100
TA100	NA	173	0	0	0	0	93
	NA	179	0	0	0	0	86
MEAN	0.0	173.7	0.0	0.0	0.0	0.0	93.0
SD	0.0	5.0	0.0	0.0	0.0	0.0	7.0

STRAIN	Positive Control**	50	10	5	1	0.5	0.1
	NA	176	170	177	193	186	169
TA100	NA	185	161	175	167	175	172
	NA	170	166	169	188	170	173
MEAN	0.0	177.0	165.7	173.7	182.7	177.0	171.3
SD	0.0	7.5	4.5	4.2	13.8	8.2	2.1

* All plates were dosed at 100 ul/plate
** Positive controls were not used in the Range Finding Assay
*** The negative control used in the assay was DMSO

TABLE VI

Confirmation Assay - Range Finding With Microsomal Activation

Technical Initiation: 11/06/92

Technical Completion: 11/09/92

Revertants/Plate*

STRAIN	CONTROLS		TEST ARTICLE				
			DOSE LEVELS (ug/plate)				
	Positive Control**	Negative Control***	100000	5000	1000	500	100
	NA	193	0	0	0	0	69
TA100	NA	186	0	0	0	0	84
	NA	184	0	0	0	0	73
MEAN	0.0	187.7	0.0	0.0	0.0	0.0	75.3
SD	0.0	4.7	0.0	0.0	0.0	0.0	7.8

STRAIN	Positive Control**	50	10	5	1	0.5	0.1
	NA	177	186	188	173	179	178
TA100	NA	177	183	191	175	174	174
	NA	169	179	178	174	180	175
MEAN	0.0	174.3	182.7	185.7	174.0	177.7	175.7
SD	0.0	4.6	3.5	6.8	1.0	3.2	2.1

* All plates were dosed at 100 ul/plate
** Positive controls were not used in the Range Finding Assay
*** The negative control used in the assay was DMSO

TABLE VII
Confirmation Assay - Reverse Mutation Assay Without Microsomal Activation

Technical Initiation: 11/12/92
Technical Completion: 11/15/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE					
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 500	50	5	0.5	0.05	0.01
	164	33	4	30	31	32	36	34
TA98	172	35	1	32	33	33	32	36
	169	36	0	34	30	33	35	35
MEAN	168.3	34.7	1.7	32.0	31.3	32.7	34.3	35.0
SD	4.0	1.5	2.1	2.0	1.5	0.6	2.1	1.0
	326	126	13	118	123	125	120	126
TA100	335	120	4	124	126	124	123	125
	340	122	9	121	120	122	123	119
MEAN	333.7	122.7	8.7	121.0	123.0	123.7	122.0	123.3
SD	7.1	3.1	4.5	3.0	3.0	1.5	1.7	3.8
	134	24	0	23	24	20	25	21
TA1535	141	20	0	21	23	21	22	24
	144	21	0	22	23	24	22	24
MEAN	139.7	21.7	0.0	22.0	23.3	21.7	23.0	23.0
SD	5.1	2.1	0.0	1.0	0.6	2.1	1.7	1.7
	100	10	0	10	11	12	13	11
TA1537	110	10	0	10	13	10	12	11
	108	12	0	10	10	11	12	12
MEAN	106.0	10.7	0.0	10.0	11.3	11.0	12.3	11.3
SD	5.3	1.2	0.0	0.0	1.5	1.0	0.6	0.6
	130	18	0	19	16	18	17	18
TA1538	126	17	0	18	17	20	18	18
	125	19	0	18	17	16	19	19
MEAN	127.0	18.0	0.0	18.3	16.7	18.0	18.0	18.3
SD	2.6	1.0	0.0	0.6	0.6	2.0	1.0	0.6

* All plates were dosed at 100 ul/plate

** The positive control used was sodium azide for strains TA-100 and TA-1535, 2-nitrofluorene for strain TA-98, and 9-aminoacridine for strain TA-1537

*** The negative control used in the assay was DMSO

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

TABLE VIII
Confirmation Assay - Reverse Mutation Assay With Microsomal Activation

Technical Initiation: 11/12/92
Technical Completion: 11/15/92

Revertants/Plate*

	CONTROLS		TEST ARTICLE					
STRAIN	Positive Control**	Negative Control***	DOSE LEVELS (ug/plate) 500	50	5	0.5	0.05	0.01
	183	39	2	43	42	44	39	42
TA98	179	40	1	40	40	40	41	42
	185	42	7	40	41	39	42	40
MEAN	182.3	40.3	3.3	41.0	41.0	41.0	40.7	41.3
SD	3.1	1.5	3.2	1.7	1.0	2.6	1.5	1.2
	452	196	12	190	183	184	191	189
TA100	446	192	10	185	192	180	193	190
	449	189	13	191	195	190	189	187
MEAN	449.0	192.3	11.7	188.7	190.0	184.7	191.0	188.7
SD	3.0	3.5	1.5	3.2	6.2	5.0	2.0	1.5
	164	27	0	26	28	26	27	25
TA1535	163	27	0	29	24	26	24	25
	159	26	1	27	27	29	26	27
MEAN	162.0	26.7	0.3	27.3	26.3	27.0	25.7	25.7
SD	2.6	0.6	0.6	1.5	2.1	1.7	1.5	1.2
	120	14	0	13	15	13	15	13
TA1537	130	15	0	12	14	12	15	12
	124	14	0	15	14	15	14	16
MEAN	124.7	14.3	0.0	13.3	14.3	13.3	14.7	13.7
SD	5.0	0.6	0.0	1.5	0.6	1.5	0.6	2.1
	130	20	1	21	18	21	23	20
TA1538	136	21	2	20	17	20	19	20
	134	23	0	18	20	19	21	20
MEAN	133.3	21.3	1.0	19.7	18.3	20.0	21.0	20.0
SD	3.1	1.5	1.0	1.5	1.5	1.0	2.0	0.0

* All plates were dosed at 100 ul/plate
** The positive control used was 2-aminoanthracene for all strains
*** The negative control used in the assay was DMSO

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

QUALITY ASSURANCE STATEMENT

SPONSOR:

ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437

TESTING LABORATORY:

Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

TEST ARTICLE:

Test Article: 1,3,3-Trinitroazetidine (TNAZ)

CAS/Code #: 97645-24-4

Lot/Batch #: Not Supplied by Sponsor

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and Management.

INSPECTIONS	QUALITY ASSURANCE INSPECTIONS	REPORTS TO MANAGEMENT	REPORTS TO STUDY DIRECTOR
SCORING	09/25/92	09/25/92	09/25/92
RAW DATA	11/17/92	11/17/92	11/17/92
FINAL REPORT	11/17/92	11/17/92	11/17/92
REVISED REPORT	12/11/92	12/11/92	12/11/92

SIGNATURE OF AUTHORIZED PERSONNEL:

Kathryn M. Balch (QBV) — 12/11/92

Kathryn M. Balch, B.A. — Date
Toxikon Quality Assurance

TOXIKON PROJECT NUMBER 92G-1263
***Salmonella typhimurium* REVERSE MUTATION ASSAY (PREINCUBATION)**
(REVISED REPORT)

REPORT REVISION 92G-1263.1

Client:

ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437

Testing Laboratory:

Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Test Article:

Test Article: 1,3,3-Trinitroazetidine (TNAZ)

CAS/Code #: 97645-24-4

Lot/Batch #: Not Supplied by Sponsor

Revision:

The following revisions were done at the request of the sponsor:

Section 3.1: P.O. Box changed from 31008 to 31009
Zip Code changed from 45431-0009 to 45437

Section 10.5: Acetone is referenced as an appropriate solvent for the test article.

Section 13.4: The use of "fresh" sample is now indicated for the confirmatory assay.

None of the above revisions affect the validity of the study.

Signatures of Authorized Personnel:

Inder J. Paika, Ph.D.
Study Director

12/11/92
Date

Darol Dodd, Ph.D.
Mantech Environmental Technology

1/22/93
Date

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